

Potassium sorbate, sodium benzoate and sodium saccharin testing in food samples by Persee/PG Scientific L600 isocratic HPLC systems

Sodium benzoate and potassium sorbate are used as antimicrobial agents in food and beverage industries as they inhibit the growth of bacteria, molds and other microorganisms in foods. Saccharin sodium is widely used food industry as artificial sweetener. Sorbic acids, benzoic acids and saccharin sodium are often added together in sweet beverages, packed meat snack, sweet snacks, etc.

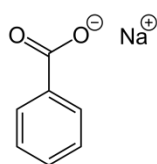


Figure 1 sodium benzoate (sodium benzoic)

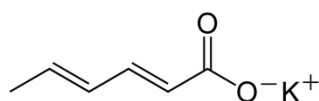


Figure 2 potassium sorbate

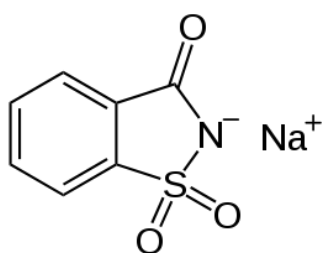


Figure 3 sodium saccharin

However, long time consumption of extra amount of such additives can lead to harmful results. Therefore, governments all around the world, for instance, CFDA in China and FDA in the US, are regulating the upper limits of all three additives. Below is the upper limits listed in FDA and CFA.

- **Sorbic acid** - PRES, GRAS/FS (Substances generally recognized as safe in foods but limited in standardized foods where the standard provides for its use.), < 0.2% - Cheeses and Cheese Rel Prods - Part 133;

PRES, GRAS/FS, < 0.3% by weight as sorbic acid, alone or comb wi potassium or sodium sorbate -Cheeses and Rel Cheese Prods - Part 133;

< 0.1% - Artificial Sweetener Fruit Jellies, Pres, and Jams - 150.141, 150.161;

< 0.2% by wt Concentrated Orange Juice -146.154;

<0.1% alone or <0.2% in combination with other preservatives - Margarine - 166.110;

GRAS, GMP - 182.3089.

- **Benzoic acid** - PRES, GRAS, 0.1%
- **Sodium saccharin:** In processed foods, in amounts not to exceed 30 milligrams of the additive, calculated as saccharin, per serving of designated size. (CFR - Code of Federal Regulations Title 21)

Persee/PG Scientific L600 HPLC system can test those three additives in one run. Below we list a method for reference.

General principle of testing

Remove fat in high-fat sample with normal hexane and precipitate protein with protein precipitator. Then, extract three chemicals with water and test them with HPLC with UV detector.

Equipment and reagent

Equipment

High pressure liquid chromatography system L600 with UV-detector, Persee/PG Scientific

Analytical balance (1mg and 0.1mg level)

Vortex

Centrifugal,

Homogenizer

Water bath

Ultra-sonic machine

Reagent

Name	Level	CAS No.
Ammonia water	GR or Better	
Potassium hexacyanferrate	AR	13943-58-3
Zinc acetate	AR	5970-45-6
Absolute ethyl alcohol	AR	67-56-1

Normal hexane	AR	110-54-3
Methyl alcohol	AR	170082-17-4
Ammonium acetate	AR	631-61-8
Formic acid	AR	64-18-6
Glacial acetic acid	AR	64-19-7

Ammonia solution (1+99): mix 1mL ammonia water into 99mL water.

Potassium ferrocyanide solution (92g/L): dissolve 106g potassium ferrocyanide into some water and constant volume to 1000mL.

Zinc acetate solution (183g/L): dissolve 220g zinc acetate into some water, add 30mL glacial acetate acid. Constant volume to 1000mL.

Ammonium acetate solution (20mmol/L): dissolve 1.54g ammonium into 1000mL water and filtrate with 0.22um membrane

Formic acid - Ammonium acetate solution (2mmol/L formic acid + 20mmol/L ammonium acetate solution): dissolve 1.54g ammonium acetate with water and add 75.2uL formic acid, then constant volume to 1000mL and filter with 022um membrane.

Standard chemicals

Sodium benzoate (CAS 532-32-1)

Potassium sorbate (CAS 590-00-1)

Saccharin sodium (CAS 128-44-9)

Standard solution making:

-Three stock solutions (1000mg/L):

Dissolve 0.118g Sodium benzoate, 0.134g Potassium sorbate and 0.117g Saccharin sodium in water and constant volume to 100mL, respectively. Store solution at 4°C.

-Combined medium solution (200mg/L):

Mix 10.0mL of each stock solution and constant volume to 50mL. Store solution at 4°C.

-Testing standard solution:

Take 0, 0.05, 0.25, 0.50, 1.00, 2.50, 5.00, 10.0mL of combined medium solution (200mg/L) and constant volume to 10mL, making standard solutions of 0, 1.00, 5.00, 10.0, 20.0, 50.0, 100, 200mg/L concentration, respectively.

Sample extraction

Take 2g of sample into centrifuge tube; add 25mL water and vortex. Ultra-sonicate it for 20min at 50°C. Set tubes to ambient.

Add 2mL Potassium ferricyanide solution and 2mL Zinc acetate solution mix well. Centrifuge tubes at 8000r/min for 5min and transfer water phase to a flask. Add water and repeat centrifuging and transferring.

Constant volume to 50mL and filter solution with 0.22um filter membrane.

-For high-fat food (chocolate, cream, fried food, etc.):

Take 2g sample to centrifuge tube and add 10mL normal hexane. Heat on water bath at 60°C for 5min. Add ammonia solution (1+99) 25mL & alcohol 1mL, vortex well. Ultra-sonicate for 20min at 50°C.

Then, add 2mL Potassium ferricyanide solution and 2mL Zinc acetate solution mix well and centrifuge at 8000r/min for 5min, same as above.

Testing parameters for HPLC:

Column: C18 column, column length 250mm, diameter 4.6mm, particle size 5µm or similar.

Mobile phase: methyl alcohol + Ammonium acetate = 5+95

Flow rate: 1mL/min

Detecting wavelength: 230nm

Sample injection 10µL

Standard solution making: inject standard solutions in HPLC and measure peak area.

Testing example:

This is the chromatograph of three peaks (sodium benzoic, potassium sorbate and Sodium saccharin, from left to right, respectively). Peaks overshadowing each other are same chemical of different concentrations (operated on Persee software for HPLC LCWIN 2.0).

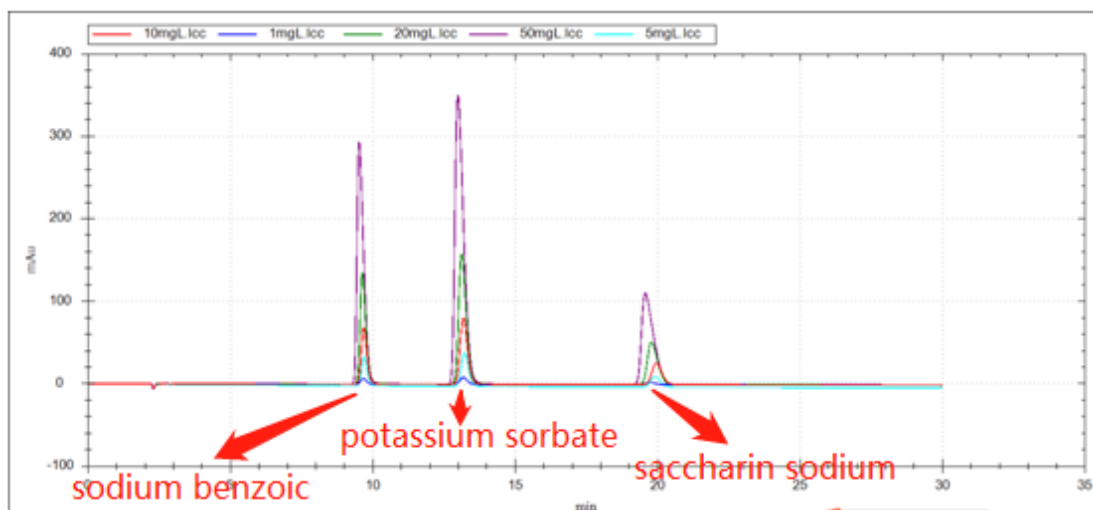


Figure 4 chromatograph of three peaks (sodium benzoic, potassium sorbate and Sodium saccharin, from left to right)

Below is the linearity figure of standard solutions of sodium benzoate. The linear ratio is 1.0000.

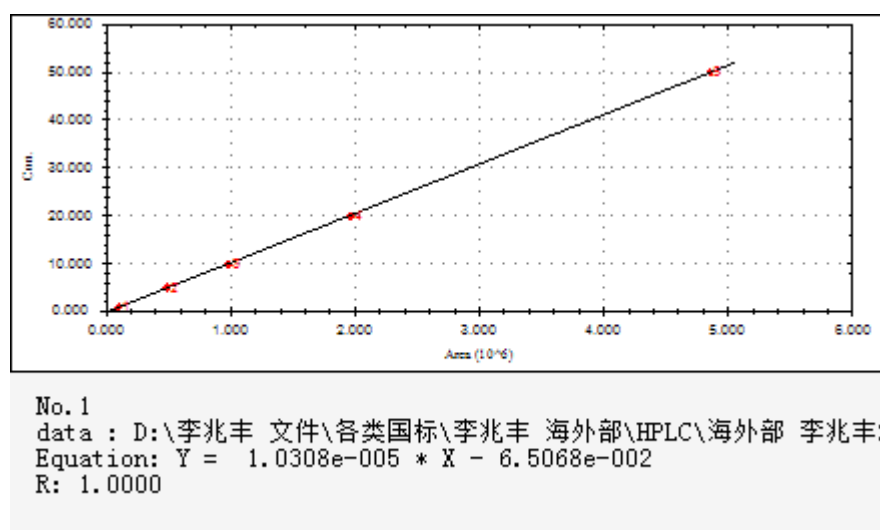


Figure 5 linearity figure of standard solutions of sodium benzoate

Shown below is the linearity figure of potassium sorbate standard solutions. The linear ratio is 1.0000.

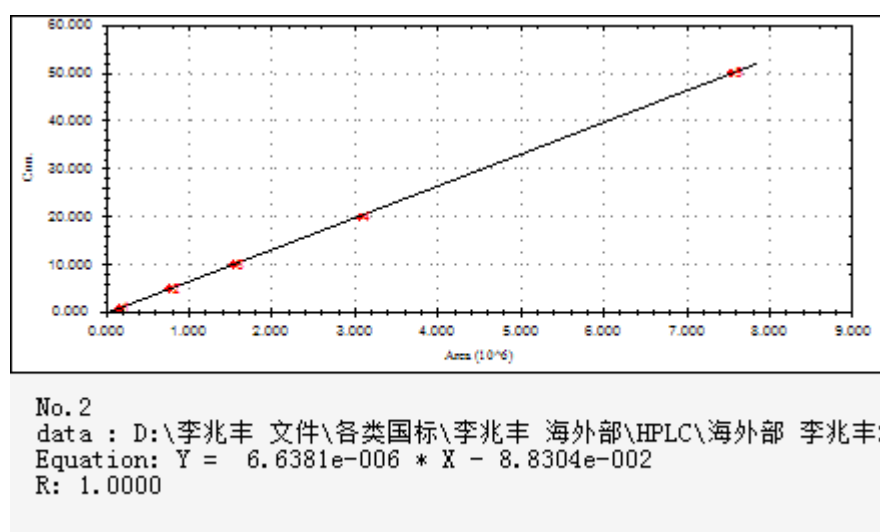


Figure 6 linearity figure of potassium sorbate standard solutions

Lastly, the linearity figure of sodium saccharin is shown below. The linear ratio is 1.0000.

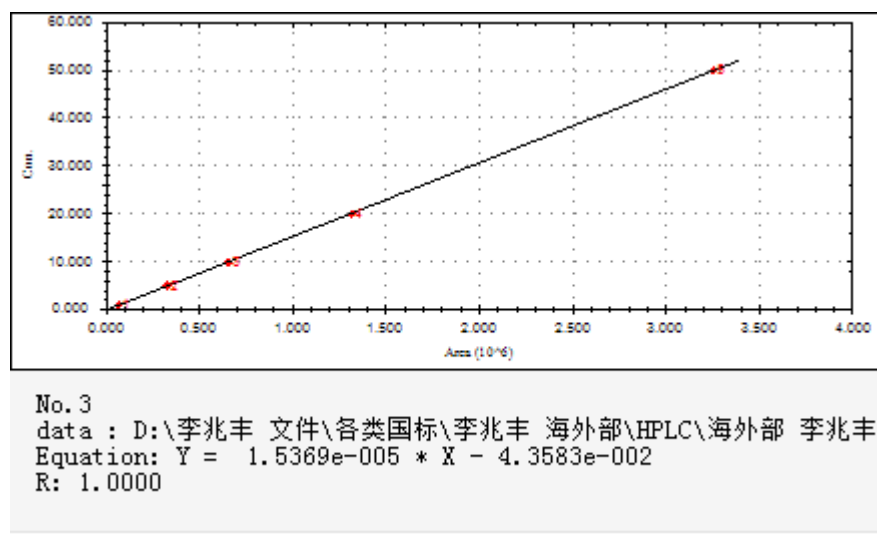


Figure 7 linearity figure of sodium saccharin is shown below

Quantitative results are shown below. As in the figures, the three chemicals present in samples were below detection limit (detection limits were around 0.2ppm).

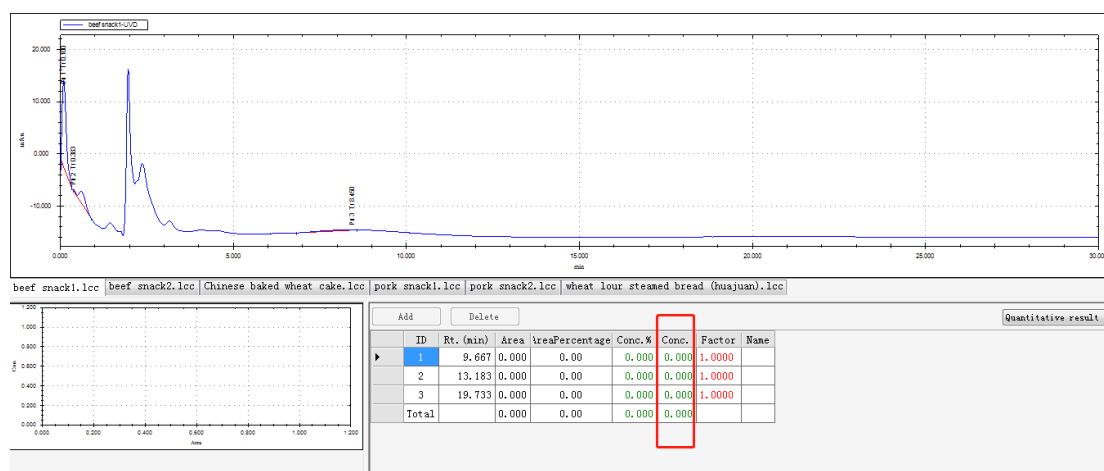


Figure 8 quantitative results of beef snack sample

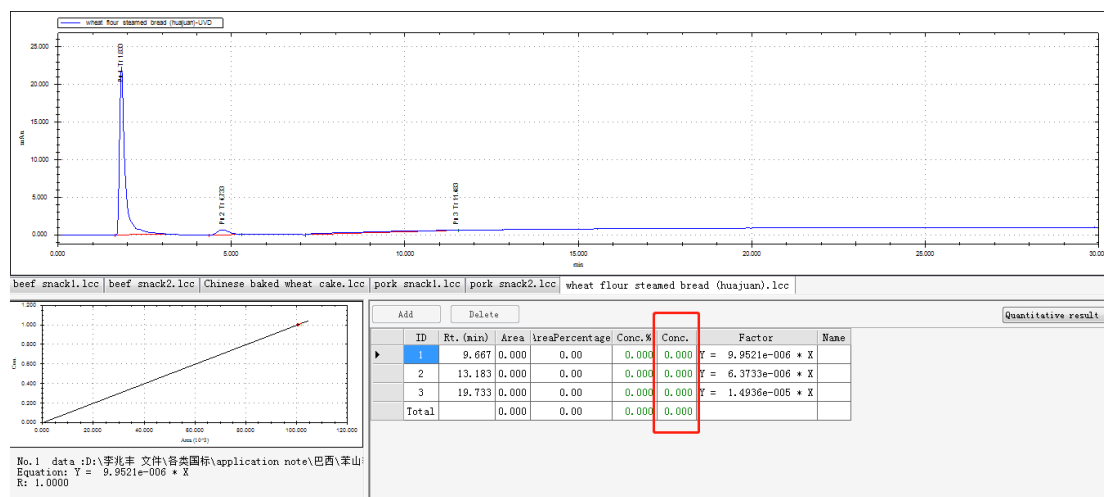


Figure 9 quantitative results of wheat flour steamed bread sample

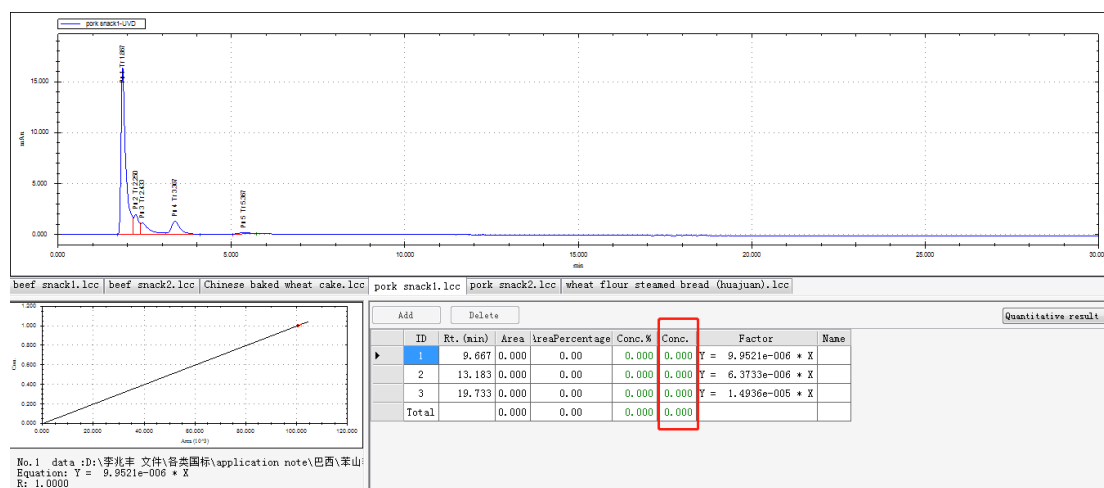


Figure 10 quantitative results of cured pork snack sample